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Somatosensory Dysfunction Following Acute Trimethyltin Exposure

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16. ABSTRACT

A variety of trimethyltin (TMT) -produced sensory and behavioral dysfunctions have been reported. In this study the functional integrity of the somatosensory system was evaluated. Animals were tested using three different measures prior to (day 0) and 1,4, and 16 days following dosing with either 0 (saline) or 7 mg/kg TMT. The tests employed were: (1) hot-plate pain threshold; (2) dorsal caudal nerve response threshold and conduction velocity; and (3) the somatosensory evoked response (SER). In the hot-plate test, TMT treated animals took longer to lick the hind paws in response to the heat. No effects were seen in the nerve function evaluation (conduction velocity and threshold), although increased N1, P1, and P2 latencies and decreased N1P1 amplitude were seen in SER recordings. The fact that no peripheral nerve function effects were observed suggests that the hot-plate and SER findings were the result of central nervous system dysfunction.

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Somatosensory Dysfunction Following Acute Trimethyltin Exposure[1]

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HOWELL, W. E., T. J. WALSH AND R. S. DYER. *Somatosensory dysfunction following acute trimethyltin exposure.* NEUROBEHAV. TOXICOL. TERATOL. 4(2) 197–201, 1982.—A variety of trimethyltin (TMT)-produced sensory and behavioral dysfunctions have been reported. In this study the functional integrity of the somatosensory system was evaluated. Animals were tested using three different measures prior to (day 0) and 1, 4, and 16 days following dosing with either 0 (saline) or 7 mg/kg TMT. The tests employed were: (1) hot-plate pain threshold; (2) dorsal caudal nerve response threshold and conduction velocity; and (3) the somatosensory evoked response (SER). In the hot-plate test, TMT treated animals took longer to lick the hind paws in response to the heat. No effects were seen in the nerve function evaluation (conduction velocity and threshold), although increased N1, P1, and P2 latencies and decreased N1P1 amplitude were seen in SER recordings. The fact that no peripheral nerve function effects were observed suggests that the hot-plate and SER findings were the result of central nervous system dysfunction.

Trimethyltin neurotoxicity Somatosensory evoked response Pain threshold Peripheral nerve function

THE neuropathpology associated with trimethyltin (TMT) exposure primarily involves the limbic system, and in particular the hippocampal CA3-CA4 pyramidal cell line [5, 9, 22]. In spite of this restrictive damage, a variety of neurobehavioral effects have been reported to follow TMT exposure. For example, acute administration of TMT produces hyperreactivity, spontaneous seizures, sensory system disturbances, and learning and memory impairments [5, 8, 10, 16, 18, 20, 23, 24]. Furthermore, we have observed that a characteristic behavior of TMT treated rats is self-mutilation restricted to the tail [8]. Several agents such as morphine [13], caffeine [4], and pemoline [17], have also been shown to produce self-mutilation. The self-mutilation following these treatments however, is not typically restricted to any particular anatomical region. The regional specificity of TMT-induced self-mutilation suggests that deafferentation of the tail might be a causative factor [14,15]. In the following series of experiments we examined the integrity of the somatosensory system at three levels of neural organization following TMT exposure: peripheral nerve function; CNS processing of sensory information; and behavioral reactivity to a painful thermal stimulus.

METHOD

Forty-two male Long-Evans hooded rats (320–540 g) anesthetized with 3.7 ml/kg Chloropent (Fort Dodge Laboratories, Inc.) were implanted with skull electrodes for recording the SER. Stainless steel jeweler screws (00-90 $\times 1/16$ in.) were threaded into the skull at 2.5 mm posterior and 2.5 mm left of bregma (primary somatosensory cortex), and 2.0 mm anterior and 2.0 mm left and right of bregma (reference and ground, respectively). Electrodes were connected to an Amphenol receptacle and encased in dental acrylic. Following surgery each animal received 100,000 units Penicillin G (IM). Animals were given a seven day postoperative recovery period before testing was begun and were individually housed in plastic cages with wood chip bedding. A 12:12 light:dark schedule with ad lib food and water was followed. The sequence of events on each test day was: (1) hot-plate test; (2) Chloropent injection; (3) nerve conduction evaluation; and (4) SER recording. Immediately following testing on day 0, animals were treated with either 0 (n=17) or 7 (n=19) mg/kg trimethyltin chloride as base by gavage and re-tested 1, 4, and 16 days after dosing.

Hot-Plate

Behavioral reactivity to thermal pain was assessed with a hot-plate method modified from Dennis *et al.* [7]. During testing, each rat was placed on a Plexiglas-surrounded slide warmer which was preheated to 51±0.5°C. The dependent measure of pain sensitivity was the latency to lick either of the hind paws. Two trials (LICK 1 and LICK 2) separated by a 180 sec intertrial interval were run, each with a 60 sec maximum duration to avoid damaging tissue.

[1]This manuscript has been reviewed by the Health Effects Research laboratory, U. S. Environmental Protection Agency and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Dorsal Caudal Nerve Conduction

Immediately following hot-plate testing animals were anesthetized with Chloropent (3.7 ml/kg) and placed into a stereotaxic frame mounted above a temperature controlled paraffin oil bath (37.5°C); pivoting the frame allowed immersion of the tail into the oil. Before the tail was immersed, three pairs of platinum needle electrodes (Grass E2B) were inserted into the tail: two stimulating and one recording. The proximal electrode of each stimulating pair was the anode. The recording pair was positioned at a distal location within the right dorsal caudal muscle. The two stimulating pairs were separated by 100±0.5 mm (cathode to cathode) and positioned proximal to the recording electrodes (cathode of distal stimulating pair 10±0.5 mm from proximal electrode of recording pair). Electrodes of each stimulating and recording pair were separated by 1.0 mm. The tail was grounded between the recording and most distal stimulating pair (5 mm distal from the cathode). The stimulating electrodes were inserted alongside the right dorsal caudal nerve, penetrating the skin surface by approximately 1 mm. Following electrode insertion, the tail was lowered into the warm oil and allowed to equilibrate for 5 min before testing was begun. Leads from the recording electrodes were connected to a high impedance amplifier with high- and low-pass filter settings at 0.3 and 10 kHz, respectively. A cable leading to an identically configured second amplifier was connected to the head socket for recording the SER. A Grass S-88 stimulator with PSIU6 constant current adapters was used for generating all stimulus pulses. Each stimulus consisted of a square wave pulse (5 Hz, 0.1 msec duration). Threshold stimulus intensity for eliciting a compound nerve action potential was determined by gradually increasing the current level until the compound nerve action potential was visually detected on an oscilloscope. The proximal stimulating pair was used to determine this threshold. This pair was then supramaximally stimulated at an intensity level 50% above that level at which any further action potential amplitude increase could no longer be detected. Thirty-two compound action potential responses were averaged with a Nicolet 1072 averager (20 μsec sampling rate). The latency of the take-off point for the first positive deflection was determined. The same procedure was then followed for the distal stimulating pair. The latency difference between these two measures and the interelectrode pair distance (100 mm) were then used to compute the mixed nerve conduction velocity (m/sec).

Somatosensory Evoked Response

The proximal stimulating electrode pair used during nerve conduction testing was used to elicit the SER (proximal electrode as cathode). The caudal nerve was stimulated at an intensity sufficient to produce a maximal compound nerve action potential, with a square pulse (1 Hz, 0.1 msec duration). Sixty-four responses were used to derive an average waveform. Major peak latencies (to the nearest 0.002 msec) and peak-to-peak amplitudes were determined.

RESULTS

Data from all three experiments were analyzed within a single repeated measures multivariate analysis of variance (MANOVA) [21]. Data from day 16 were not included in the analysis because of head plug pulls and TMT related mortality. Difference scores (Day X − Day 0) derived from collected data were used in all analyses. Pillai's Trace indicated a significant main effect of dose, $F(13,21)=3.64$, $p<0.0043$, and a significant dose by day interaction, $F(13,17)=3.10$, $p<0.0156$. Individual variables were subsequently assessed using univariate ANOVAs.

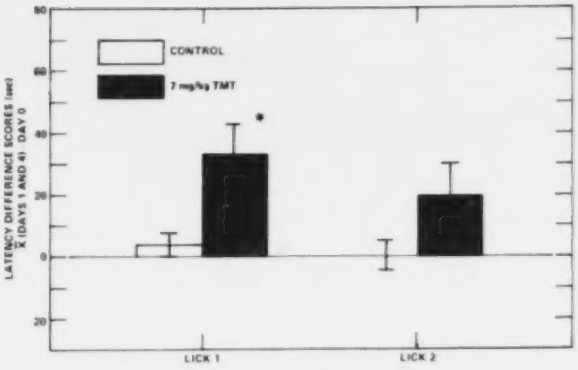

FIG. 1. Difference scores for latency to hind-paw lick $\bar{X}$(Days 1 and 4) − Day 0 (Means±SE). *Significantly different from control $p<0.005$.

Hot-Plate

TMT significantly increased latencies to hind-paw lick during the first (LICK 1), $F(1,65)=7.54$, $p<0.0097$, but not the second (LICK 2) test session (Fig. 1). No dose by day interactions were observed for either variable.

Dorsal Caudal Nerve Conduction

TMT exposure produced no significant changes in either conduction velocity or stimulus intensity threshold for eliciting the compound nerve action potential (Table 1).

Somatosensory Evoked Response

The SER elicited by caudal nerve stimulation consisted of three negative peaks at approximately 11, 18, and 42 msec, and two positive peaks at 14 and 23 msec. Schematic SERs using the mean latencies and amplitudes for the control and TMT groups on Day 4 are plotted in Fig. 2.

In general, TMT increased SER latencies and decreased SER amplitudes. ANOVAs demonstrated significant dose by day interactions for N1 and P1 latencies (Fig. 3A) and N1P1 amplitude (Fig. 4), $F's(1,29)>12.40$, $p's<0.0014$; while a significant main effect of dose was observed for P2 latency (Fig. 3B), $F(1,29)=5.88$, $p<0.0200$. Within day analysis revealed that N1 and P1 latencies were significantly increased, $F's(1,29)>6.41$, $p's<0.0170$, and N1P1 amplitude was decreased, $F(1,29)=8.74$, $p<0.0061$, on day 4.

DISCUSSION

The results demonstrate that exposure to TMT produces somatosensory dysfunction. The absence of effects on measures of peripheral mixed nerve function suggests that the observed hot-plate and SER changes were the result of central nervous system impairment. This hypothesis might receive further indirect support from the observation that while TMT increases hot-plate latencies it does not alter

TABLE 1

MIXED NERVE CONDUCTION VELOCITY (m/sec) AND STIMULUS INTENSITY LEVEL THRESHOLD (mA) FOR ELICITING A COMPOUND NERVE ACTION POTENTIAL FOLLOWING 7 mg/kg TRIMETHYLTIN (TMT) EXPOSURE*

	Dose	$\bar{X}$ Days 0, 1 and 4	$\bar{X}$ Difference Score $\bar{X}$ (Days 1 and 4) − Day 0
Conduction Velocity (m/sec) $F(1,65)=1.72$ $p=0.1985$	Control N=33	129.28 ± 1.23	0.90 ± 0.61
	7 mg/kg N=33	130.53 ± 0.32	−0.32 ± 0.50
Threshold (mA) $F(1,65)=2.21$ $p=0.1463$	Control N=33	2.01 ± 0.01	0.13 ± 0.08
	7 mg/kg N=33	2.05 ± 0.01	−0.13 ± 0.10

*Means ± SE; no significant treatment effects.

Conduction velocity values (m/sec) were determined using the compound nerve action potential elicited by supramaximal electrical stimulation of the dorsal caudal nerve. The minimum stimulus intensity level (mA) necessary to visibly detect the nerve action potential on an oscilloscope was used as the threshold value.

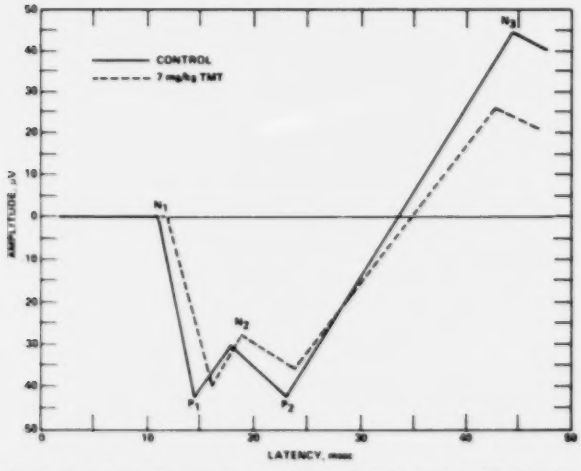

FIG. 2. Schematic somatosensory evoked potentials (SER) with mean (Day 4) peak latencies and amplitudes plotted for control and TMT treated groups.

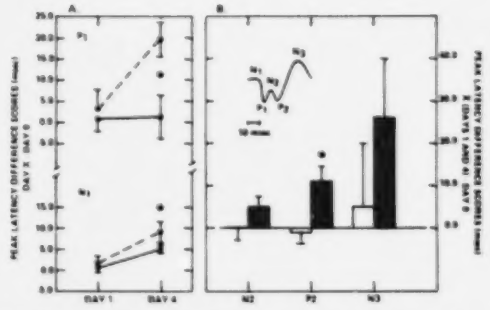

FIG. 3. Effects of 7 mg/kg TMT on peak latencies of the SER elicited by electrical stimulation of the dorsal caudal nerve. (A) N1 and P1 latency difference scores for days 1 and 4 (Day X–Day 0); a dose by day interaction with significance on day 4 for each was observed (solid line=control; dashed line=TMT treated). (B) Difference scores for remaining SER peak latencies, $\bar{X}$(Days 1 and 4)−Day 0), with a significant main effect of dose for P2 latency. Positive difference scores in Figs. 3A and 3B represent increased latencies (Means±SE) (open bars=control; cross-hatched bars=TMT-treated). *Significantly different from control $p<0.02$.

flinch-jump responses to electric shock [23]. It has been suggested by a number of investigators that these two measures of pain sensitivity are organized at different levels of the neuraxis, with flinch-jump responses mediated at a spinal reflex level and hot-plate responses at a more central integrative level [2,6]. Therefore, the data from the present experiment do support a central (at least supraspinal), and not peripheral somatosensory system locus of action for TMT.

The early components of the SER (N1-P1-N2) are generally thought to represent the arrival of input to primary somatosensory cortex via specific somatosensory pathways of the lemniscal system. This system is primarily concerned with the mediation of touch and kinesthesis. The later components of the SER (P2-N3) are thought to be produced by inputs to somatosensory cortex from non-specific, extralemniscal brain regions [1,19]. Specific somatosensory pathway input is transmitted via long, large diameter nerve fibers, whereas shorter, small diameter fibers are found in the nonspecific pathways [11]. Since both early (N1 and P1) and late (P2) component peak latencies were increased, it is likely that TMT influences both specific and nonspecific somatosensory systems. These latency increases represent either: (1) decreased axonal conduction velocity along somatosensory pathways within the CNS; (2) increased

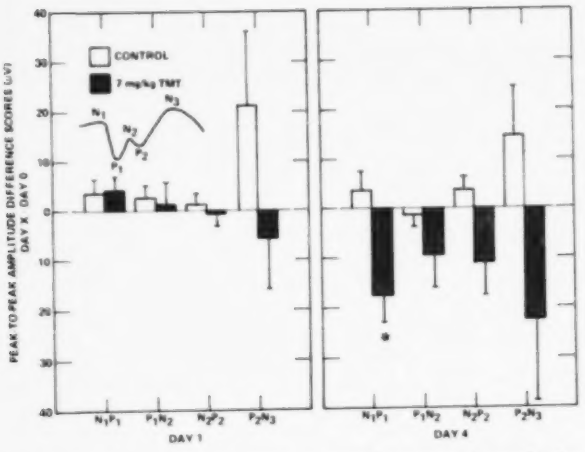

FIG. 4. Effects of 7 mg/kg TMT on SER peak-to-peak amplitudes recorded 1 and 4 days following exposure. Positive difference scores (Day X − Day 0) represent increased amplitudes (Means ± SE). *Significantly different from control $p<0.01$.

synaptic delay; or (3) non-synchronous timing of somatosensory information within the CNS.

A CNS axonal conduction velocity decrease could be produced by axonal demyelination. Such a mechanism is not supported by the fact that no velocity change was evident in the caudal nerve, nor have there been any reports of demyelination following TMT exposure. Loss of cells with fast conducting axons i.e., large neurons with large diameter axons, or the degeneration of these axons within somatosensory pathways, would also increase SER latencies. Although there have been no reports of TMT related pathology within somatosensory systems, TMT does produce damage within the limbic system [5, 9, 22]. Of particular interest is the observation that in the rat hippocampus, the largest neurons are among the most affected [9]. A loss of neurons might also account for the decreased N1P1 amplitude.

Potential causes for an increased synaptic delay include disruption of transmitter availability, release, or postsynaptic receptor binding. A decrease in synaptosomal uptake of gamma-aminobutyric acid (GABA) following TMT exposure has been reported [22]. Non-synchronous timing of somatosensory information within the CNS could be a by-product of such synaptic impairment. Any influence on the dispersion of nerve impulses elicited by somatosensory input might serve to temporally broaden components of the SER; this in turn might increase the temporal separation of these components and decrease their corresponding peak amplitudes (such as the observed N1P1 amplitude decrease). To test this we compared the mean time (±SE) between N1 and N2 for the control and TMT treated groups; there was no significant difference for this measure (71.8±3.0 msec vs 70.4±2.4 msec, respectively). Thus, if observed latency increases are due to synaptic delay, this delay does not appear to influence the timing of somatosensory events within the CNS.

The P2 peak latency increase suggests that TMT influences non-specific somatosensory systems. The increased pain threshold or decrease in reactivity to pain demonstrated by the hot-plate experiment may be the behavioral counterpart of this electrophysiological finding. The reticular formation has been associated with processes of pain perception, as well as the generation of the late SER components (such as P2) [1, 3, 19]. However, in light of the fact that barbiturate anesthesia suppresses the reticular activating system [12], the contribution of this system to the SER should be questioned. It is more likely that TMT damage occurred in other non-specific somatosensory regions, such as the lateral spinothalamic tract which also mediates pain.

In conclusion, somatic sensation from the tail is clearly altered but not eliminated by TMT exposure. The question therefore remains whether the self-mutilation produced by TMT is related to this sensory dysfunction. SER findings suggest that the origin of the dysfunction is probably central and that cell loss within specific and nonspecific somatosensory pathways may be involved.

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